THE RELATION OF ALLANTOIN EXCRETION TO LEUKOPENIA AND LEUKOCYTOSIS IN RABBITS *

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It has been known for many years[1] that the intravenous injection of the products of protein degeneration or of certain bacteria or their autolysates causes a very prompt and marked leukopenia in the peripheral circulation, followed in a few hours by a considerable leukocytosis. Later studies[2] have led to the conclusion that the leukopenia is the result of the accumulation of great numbers of polymorphonuclear leukocytes in the internal circulation, especially in the liver, spleen, and lungs. There has been, however, no explanation of the purpose of this localization of leukocytes except that of Bull,[3] who ascribes the removal of living typhoid organisms introduced into the circulation to phagocytosis by the polymorphonuclear cells accumulated in the central organs. This explanation is not entirely satisfactory. Nor is it known whether these cells eventually re-enter the general blood stream to take part in the leukocytosis which follows the leukopenia or whether they are all or in part destroyed in the internal circulation. There is some little evidence that tends to the belief that at least some of the leukocytes are destroyed. For example, in several of our experiments examination of the internal organs at the low point of the leukopenia failed to reveal either in smears or sections the usual accumulation of leukocytes. Again the Arneth count of the polymorphonuclear leukocytes made during the period of leukocytosis shows so great an increase in the percentage of young forms as to suggest that at least a part of the matured forms had not returned to the circulation. It must be remembered, however, that both the leukopenia and the leukocytosis are the result very largely of changes in the number of polymorphonuclear leukocytes in the peripheral blood.

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[1] Löwit: Studien zur Physiologie und Pathologie des Blutes und der Lymph, 1892.
[2] Goldscheider and Jacob: Ztschr. f. klin. Med., 1894, 25, p. 373.
[3] Jour. Exper. Med., 1915, 22, p. 475.

This question of the fate of the leukocytes is of importance at the present time on account of its bearing on the recently advanced treatment of various infections by injections of nonspecific vaccines or even of pure proteoses. It seemed to us possible in view of the magnitude of the changes in the leukocyte count that quantitative determination of the excretion of the end products of nucleic-acid metabolism might throw some light on this problem or at least show variations which could be correlated with the changes in the leukocyte count.

The relation of the end products of nucleic-acid metabolism to leukocytosis and to leukocytic destruction is still unsettled. Horbaczewski first advanced the theory that ingested substances increase the excretion of uric acid only in proportion to the leukocytosis they excite, and he believed that dead leukocytes were the source of the uric acid. On the other hand, Plimmer, Dick and Lieb[4] observed that the increase in the excretion of the uric acid after the ingestion of substances causing leukocytosis is present only during the increase of leukocytes in the blood, and they advanced the theory that the uric acid is a product of the metabolism of living leukocytes. Certainly some definite relation does exist and it has often been shown that an increased excretion of uric acid occurs after the ingestion of nucleic acid and in leukemia, but it must be remembered that a similar increase occurs in fever and in many other pathologic disturbances.

METHODS

Rabbits were used and these were kept in metabolic cages on a constant daily diet of 200 gm. of carrots, which they ate completely even on the days of treatment. Catheterization was carried out daily just before feeding, except when noted otherwise in the tables. Injections were given in the marginal ear vein and blood counts were taken from the veins of the other ear. Typhoid bacilli were used for injection on account of the uniformity of leukocytic reaction which has been shown to follow intravenous introduction. Cultures from a stock strain of Bacillus typhosus were made on agar slants the day before injection and grown at 30 C. for 24 hours. The growth was washed off in salt solution, and if killed, was heated at 60 C. for 1 hour. The number injected was merely estimated roughly as from one-tenth to one-half of a slant. The blood counts were made according to the usual technic. All injections were given about 4 hours after feeding.

The 24-hour urine was collected under toluol. Total nitrogen was determined by the Gunning-Kjeldahl method and urea by the urease method and by Benedict's method. According to the suggestion of Plimmer and Skelton,[5] the difference between the figures obtained by these two methods was accepted as representing the greater part of the allantoin nitrogen. It has been found

[4] Jour. Physiol., 1909, 39, p. 98.
[5] Biochem. Jour., 1914, 8, p. 70.

by Taylor and Adolph[6] and others that Benedict's method of estimating the amount of urea includes with the urea about 70-75% of the allantoin present. In the rabbit allantoin represents the chief end product of nucleic-acid metabolism, and we have considered the allantoin fraction obtained by difference between the two determinations of urea as an index of nucleic-acid metabolism and have used the uncorrected figures throughout. The tables therefore record the total nitrogen, the urea nitrogen (urease method), and the allantoin nitrogen (difference between the determinations by the Benedict and by the urease methods).

RESULTS AFTER INJECTION OF KILLED TYPHOID BACILLI

Table 1 gives the results in 3 of the rabbits injected with killed organisms. In each of these animals the leukocytic reaction was prompt and definite, and tho the counts were not made at very frequent intervals, yet those recorded show both a definite leukopenia and a subsequent leukocytosis. It is of course probable that the extreme in either was not obtained. In Rabbit 3 the high level of allantoin excretion is possibly to be explained as due to an infection with "snuffles," which was present in this rabbit to a mild degree, becoming more severe toward the end of the period. In none of these animals was there any marked change in the allantoin figures following the injection.

RESULTS AFTER INJECTION OF LIVING TYPHOID BACILLI

The results obtained after the injection of live organisms were much more variable. Examples are given in Table 2. In some instances (Rabbit 6) the allantoin fraction showed no more variation than in the animals injected with killed organisms. On the other hand, in some animals with no greater leukocytic reaction there occurred a marked rise in the total nitrogen figure, which was chiefly due to an enormous increase in the allantoin fraction. Thus, in Rabbit 4 the previously high allantoin figure was doubled the day following the injection and in Rabbit 5 the increase was from 42 to 448 mg. In the latter animal this increase did not appear until the day following the injection and we failed to obtain a count showing leukocytosis until 3 days later. It is probable, however, that the usual leukocytic crisis did occur and was missed as a result of the infrequency of the counts taken. This animal was never catheterized, but the increase in the allantoin figure is too great to be explained by any such factor. Rabbits 4 and 6 had been previously immunized with 3 injections of killed bacilli given at 2-day intervals in the hope of obtaining a greater

[6] Jour. Biol. Chem., 1914, 18, p. 521.

TABLE 1

ALLANTOIN EXCRETION IN RELATION TO LEUKOPENIA AND LEUKOCYTOSIS FOLLOWING THE INJECTION OF KILLED TYPHOID BACILLI INTO RABBITS

Day	Weight, Gm.	Urine, c.c.	Nitrogen			Leukocytes
			Total	Urea	Allantoin	
			RABBIT 1 *			
1	....	95	.158	.136	.022	
2	....	115	.130	.092	.038	
3	....	105	.128	.098	.030	
4	....	98	.109	.098	.011	
5	1100	58	.075	.049	.026	Before injection,† 5800 1 hour after injection, 2100
6	....	130	.125	.092	.033	24 hours after injection, 24,000
7	950	60	.055	.049	.006	
			RABBIT 2			
1	....	155	.449	.299	.095	
2	....	160	.579	.326	.134	
3	....	140	.435	.272	.090	
4	....	158	.443	.250	.082	
5	1485	152	.457	.266	.096	Before injection,† 15,900 1 hour after injection, 2400
6	....	140	.370	.223	.072	26 hours after injection, 43,000
7	1430	Urine lost				50 hours after injection, 13,800
8	....	Urine lost				
9	1320	130	.481	.265	.070	
			RABBIT 3			
1	1760	100	.575	.218	.299	
2	1790	145	.554	.187	.281	
3	1790	150	.523	.229	.234	
4	1775	175	.484	.203	.203	
5	1735	180	.377	.140	.237	Before injection,† 13,600 1 hour after injection, 4000 19 hours after injection, 50,000
6	1645	110	.671	.229	.327	24 hours after injection, 108,400
7	1635	145	.733	.300	.353	48 hours after injection, 28,200
8	1640	153	.751	.338	.320	72 hours after injection, 8100
9	....	...	.....	.....	.....	Severe snuffles

* Not catheterized.

† Rabbits 1, 2, and 3 each received an injection of one-tenth slant of killed typhoid bacilli on the 5th day.

TABLE 2

ALLANTOIN EXCRETION IN RELATION TO LEUKOPENIA AND LEUKOCYTOSIS FOLLOWING THE INJECTION OF LIVING TYPHOID BACILLI INTO RABBITS

Day	Weight, Gm.	Urine, c.c.	Nitrogen			Leukocytes
			Total	Urea	Allantoin	
RABBIT 4						
1	....	130	.434	.130	.255	
2	....	155	.528	.218	.251	
3	....	125	.577	.208	.304	
4	1665	170	.598	.213	.341	
5	....	200	.996	.333	.648	Before injection,† 10,500 1 hour after, 2700 21 hours after, 36,000
6	....	180	.869	.348	.472	24 hours after, 11,300
7	1510	150	.725	.229	.450	48 hours after, 14,200
8	1515	170	.803	.255	.509	
9	....	160	1.134	.442	.619	
10	....	190	1.053	.332	.643	
11	1430	82	.648	.224	.390	Diarrhea; W. B. C. 11,400
12	1470	90	.434	.142	.261	
13	1520	90	.322	.111	.159	
14	1570	135	.252	.083	.143	
15	1560	162	.393	.104	.242	
RABBIT 5 *						
1	....	...	.....	.....	.....	
2	1280	75	.326	.207	.024	
3	....	80	.316	.234	.019	
4	....	50	.245	.141	.049	
5	....	80	348	.246	.042	Before injection,† 7000 1 hour after, 3600
6	....	100	.903	370	.448	24 hours after, 6700
7	1140	45	.351	.190	.115	
8	....	100	1.100	.751	.244	22,300
9	Sick—killed		.....	.....	.....	
RABBIT 6						
1	....	140	.382	.130	.156	
2	....	125	.390	.187	.151	
3	....	130	.447	.156	.221	
4	1397	138	.432	.146	.178	
5	....	100	.426	.151	.144	Before injection,† 12,200 1 hour after, 2800 21 hours after, 33,300
6	....	175	.426	.151	.179	24 hours after, 20,300
7	1320	80	.270	.078	.174	48 hours after, 11,700

* Not catheterized.

† Rabbits 4, 5, and 6 each received injections of living typhoid bacilli on the 5th day, Rabbits 4 and 6 receiving one-tenth slant, and Rabbit 5, one-half slant. Rabbits 4 and 6 had previously been immunized with 3 doses of killed organisms at 2-day intervals.

leukocytosis, as described by Gay and Claypoole.[7] However, no hyperleukocytosis was obtained and these animals reacted similarly to the others.

DISCUSSION

Concerning the animals injected with dead organisms it must be concluded that no light is shed by this study either on the fate of the leukocytes driven into the internal circulation or on the relation of allantoin excretion to the leukopenia and leukocytosis brought about by such injections.

In the animals given injections of living organisms the leukocytic reactions, at least in the peripheral blood, are the same as when dead organisms are injected, so that an additional factor must be invoked to explain the sudden increase in the nitrogen metabolism and especially in the allantoin fraction. Two possibilities suggest themselves. First, there is a possiblity that when live organisms are injected there occurs a destruction of leukocytes in the internal circulation as a result of their activity in taking up the injected bacilli and that this does not occur when heated cultures are employed. On the other hand, there is the second possibility that the changes in excretion are independent of the leukocytic reaction altogether and dependent on some other factor, such as fever, which in turn is induced only by the living organisms. This is made probable by the figures observed in a rabbit not detailed in this report which developed severe snuffles during a preliminary control period. This animal exhibited an abrupt rise of total nitrogen and of the allantoin fraction similar to that obtained following the injection of living organisms. It is impossible, however, to arrive at any positive conclusion on this point until further investigation shall have been made.

CONCLUSIONS

Injection of typhoid bacilli either living or dead into the peripheral circulation of the rabbit causes a leukopenia and subsequently a leukocytosis.

After injections of dead organisms there occurs no alteration in the nitrogenous excretion, as shown in determinations of total nitrogen, urea nitrogen, and allantoin nitrogen.

After injections of living organisms there frequently occurs an abrupt increase in nitrogenous excretion, which is chiefly due to a marked increase in the allantoin fraction.

[7] Arch. Int. Med., 1914, 14, p. 662.